HED DOC. NO. 014147

May 09, 2000

MEMORANDUM

SUBJECT: ***VINCLOZOLIN*** - Report of the FQPA Safety Factor Committee Regarding Recommendations for Cancer Risk Assessment.

FROM: Brenda Tarplee, Executive Secretary
FQPA Safety Factor Committee
Health Effects Division (7509C)

THROUGH: Ed Zager, Chairman
FQPA Safety Factor Committee
Health Effects Division (7509C)

TO: William Hazel, Risk Assessor
Reregistration Action Branch 1
Health Effects Division (7509C)

PC Code: 113201

The Health Effects Division (HED) FQPA Safety Factor Committee (SFC) met on May 1, 2000 to discuss the applicability of the FQPA safety factor for vinclozolin to the non-linear approach for quantitation of potential human cancer risk. The key concern for infants and children exposed to vinclozolin is the potential for developmental/reproductive effects related to the anti-androgenic properties of this chemical. The FQPA SFC concluded that the Chronic Population Adjusted Dose (cPAD) would be protective against both potential carcinogenic effects and developmental/reproductive effects.

I. BACKGROUND

(*DRAFT Memorandum:* S. Diwan to M. Metzger, W. Hazel, and D. Anderson received by email, April 26, 2000)

In 1997 the HED Cancer Peer Review Committee classified Vinclozolin as a "Group C-human carcinogen" based on the increase in the incidence of testicular Leydig cell tumors in rats and supported by the increased incidence of testicular Leydig cell hyperplasia in mice. The CPRC concluded that the available data for vinclozolin appear to demonstrate that anti-androgenic activity is the mode of action for testicular Leydig cell tumor formation. The CPRC recommended that for the purposes of dose-response assessment and characterization, a non-linear approach using margin of exposure (MOE) based on a NOAEL for anti-androgenic-related effects should be used for quantitation of potential human cancer risk. In addition, the CPRC recommended the toxicity endpoint selected by the Toxicity Endpoint Selection Committee be utilized. This toxicity endpoint is based on a decrease in epididymal weight at 30 mg/kg/day seen in a 2-generation study. The NOAEL for risk assessment is 4.9 mg/kg/day (Cancer Peer Review Committee Report on the 1/15/97 meeting for Vinclozolin).

As part of the Reregistration eligibility evaluation for vinclozolin, HED was asked to determine the potential cancer risk for infants and children exposed to this pesticide. A meeting among a select group of HED scientists was held to discuss the issue of anti-androgenic effect on the formation of testicular Leydig cell tumors in rats treated with vinclozolin, and the implications for potential risk to infants and children. A key question was whether or not this mode of action would result in testicular Leydig cell tumors in children. The HED group agreed that the possibility of tumor development later in life resulting from childhood exposure could not be discounted, although formation of tumors in children was unlikely (Kaplan et al., 1986; Grapin et al., 1994; Jimenez et al., 1996). It was also agreed that if the toxicity endpoint for anti-androgenic effects, precursors to tumor and hyperplasia formation, were used for cancer risk assessment, it would be protective for tumor formation which occurred at higher dose levels than the anti-androgenic effects. This group noted that a decrease in prostate weight was seen in rats which received vinclozolin at doses as low as 6 mg/kg in a perinatal rat developmental toxicity study (Gray et al., 1999). The NOAEL for this effect was 3 mg/kg. After considering all of the available data, the HED group concluded that it is more reasonable to base the toxicity endpoint for cancer risk assessment on the effects in the Gray et. al. study (decrease in prostate weight at **.** 6 mg/kg) and the dose for risk assessment at 3 mg/kg.

On April 19, 2000, the HED Cancer Assessment Review Committee (CARC) considered the proposal that the anti-androgenic endpoint (decreased prostate weight) and that the point of departure (POD) for risk assessment be established at the NOAEL of 3 mg/kgbw/day. The CARC accepted the proposal and concluded that infants, children, and adults are protected from testicular Leydig cell tumors (TLCT) through a non-linear assessment with a point of departure of 3 mg/kg/day and a margin of exposure (MOE) of 1000 (10X for intraspecies extrapolation; 10X for interspecies variation; and 10X for FQPA - see Attachment 1.). The CARC concluded that the mode of action for vinclozolin-related effects in the fetus, infants, children, and adults is inhibition of androgen receptors. In addition

they concluded: (1) that the tumor, developmental and reproductive effects are related to the anti-androgen effects of vinclozolin; (2) that the testicular Leydig hyperplasia (TLCH) seen in a reproduction study are probably precursors of adenomas; (3) that a NOAEL of 3 mg/kg/day and a LOAEL of 6 mg/kg/day, based on the lowest biological anti-androgen response seen in developmental toxicity studies, would be protective of TLCH and TLCT; and (4) that at lower dose levels, potential effects could not be distinguished from the normal variation in androgenization of male and female fetuses *in utero*. Therefore, given the commonality in the mode of action, a point of departure (POD) on anti-androgenic effects should be protective of both cancer and reproductive/developmental consequences.

II. FQPA SAFETY FACTOR COMMITTEE DELIBERATIONS

On May 1, 2000, the HED FQPA Safety Factor Committee (SFC) met to discuss the applicability of the FQPA safety factor for vinclozolin to the non-linear cancer risk assessment. This meeting was also attended by the OPP Senior Science Advisor, Penny Fenner-Crisp; the Director of the HED, Margaret Stasikowski, the HED Senior Science Advisors, Vicki Dellarco and Karl Baetcke; and other HED scientific specialists (list of attendees attached). The conclusions of the HED group and the CARC were presented to the Committee by the toxicology reviewer for vinclozolin, David Anderson.

The following key points were considered by the FQPA SFC:

1. The cancer end point of concern related to vinclozolin exposure is the formation of testicular Leydig cell tumors.
2. The weight of evidence indicates it is biologically plausible that the anti-androgenic effects of vinclozolin lead to the formation of the Leydig cell hyperplasia/tumors in rodents.
3. The mode of action for vinclozolin and some of its metabolites is considered to include competitive binding to androgen receptors, thus reducing binding of the natural androgen ligand. This results in an increased release of leutinizing hormone from the pituitary, which in turn stimulates testosterone production in testicular Leydig cells, leading to Leydig cell hyperplasia by an unknown mechanism.
4. The same anti-androgenic mode of action is indicated for both the developmental/ reproductive effects of concern and the Leydig cell tumor formation observed with vinclozolin in rodents.
5. Because the optimal hormonal environment for formation of these tumors occurs predominantly in adult animals following extended treatment with vinclozolin, there should be little concern for formation of these tumors during childhood.
6. Although Leydig cell tumors are extremely rare in children, the possibility of increased incidence of testicular Leydig cell tumors in adults resulting from prenatal and/or childhood exposure to vinclozolin cannot be ruled out (as suggested by the results of the 2-generation reproduction study wherein an increase in incidence of the same type of lesion is seen from dosing rats *in*

utero through sexual maturity into adulthood and from dosing young adult rats for 22-28 weeks - P_0 parental generation).

7. The FQPA safety factor is retained at a full 10X due to evidence of increased susceptibility following *in utero* exposure to vinclozolin in the prenatal developmental toxicity study in rats (anti-androgen related effects, such as reduced anogenital distance, areola/nipple development, and ventral prostate weight decrease in males, which occur at lower dose levels than other toxic effects seen in dams dosed at the same dose levels and time period); and since a developmental neurotoxicity study in rats with an expanded protocol is required for vinclozolin due to concern for the anti-androgenic properties of vinclozolin and its metabolites (HED Doc. No. 013895).
8. Depending on the protocol agreed upon for the required developmental neurotoxicity study, the results of this study could impact the point of departure (i.e., NOAEL) for the anti-androgenic activity of vinclozolin. This would be the case if anti-androgen-related effects are measured and found to occur at lower dose levels than those observed in the existing developmental/reproductive studies with vinclozolin.
9. The Chronic Reference Dose (RfD) for vinclozolin is derived using a NOAEL of 1.2 mg/kg/day based on the combined results of a Chronic Toxicity study in rats and a Combined Chronic Toxicity/Carcinogenicity study in rats. The LOAEL is 2.3 mg/kg/day based on foam cell aggregates in the lungs (males), eosinophilic foci in the liver (males), interstitial cell lipidosis in the ovaries (females), and lenticular degeneration of the eyes (both sexes). Other effects observed include: lesions such as edema; diffuse tubular atrophy; tubular calcification; cystic rete testis and hyperplastic rete testis; azoospermia and oligospermia in the epididymides; degenerative lesions of the accessory reproductive organs in males; interstitial lipidosis of the ovary and adrenal glands; liver cell hypertrophy; lenticular calcification; vacuolated acinar cells of the pancreas; and focal fiber atrophy of the skeletal muscle (HED Doc. No. 013919). A number of these effects also appear to occur as a result of the anti-androgenic characteristics of vinclozolin.
10. The testicular Leydig hyperplasia seen in the reproduction study (and considered to be likely precursors of the testicular Leydig cell tumors) occurred at 6 mg/kg/day. The NOAEL for these effects (proposed as the point of departure for cancer risk assessment) is 3 mg/kg/day which is higher than the NOAEL used to derive the Chronic RfD.

III. CONCLUSIONS

The FQPA SFC agreed with the HED group and the CARC that the key concern for infants and children exposed to vinclozolin is the potential for developmental/reproductive effects related to the

anti-androgenic properties of this chemical. The Committee members also agreed that the possibility of increased incidence of testicular Leydig cell tumors in adults resulting from infant and children exposure to vinclozolin cannot be ruled out. However, the Committee members reasoned that, because of the relationship between vinclozolin's anti-androgenic properties and its carcinogenic effects, protecting against the anti-androgenic effects (i.e., the mode of action) would also be protective against potential carcinogenic effects to all population subgroups (including infants and children).

Accordingly, the FQPA SFC concluded that the Chronic Population Adjusted Dose would be protective against both potential carcinogenic effects and developmental/reproductive effects. The cPAD incorporates the full, additional FQPA 10X safety factor for the protection of infants and children (i.e., it is derived from the NOAEL of 1.2 mg/kg/day with a composite uncertainty / safety factor of 1000 - 10X for intraspecies extrapolation; 10X for interspecies variation; and 10X for FQPA - see Attachment 1). Because this approach (using the cPAD) would be more protective than the proposed POD for cancer risk assessment of 3 mg/kg/day, and includes an additional 10X factor for the protection of infants and children, a separate non-linear risk assessment for cancer is not necessary.

ATTACHMENT 1.

HED DOC. NO. 013895

December 15, 1999

MEMORANDUM

SUBJECT: ***VINCLOZOLIN*** - Reassessment Report of the FQPA Safety Factor Committee.

NOTE: THIS REPORT REPLACES THE PREVIOUS REPORT OF THE FQPA SAFETY FACTOR COMMITTEE DATED MARCH 30, 1999 (HED Doc. No. 013288).

FROM: Brenda Tarplee, Executive Secretary
FQPA Safety Factor Committee
Health Effects Division (7509C)

THROUGH: Ed Zager, Chairman
FQPA Safety Factor Committee
Health Effects Division (7509C)

TO: William Hazel, Risk Assessor
Reregistration Action Branch 1
Health Effects Division (7509C)

PC Code: 113201

The Health Effects Division (HED) FQPA Safety Factor Committee (SFC) met on November 22, 1999 to re-evaluate the hazard and exposure data for Vinclozolin and maintained that the FQPA safety factor (as required by the Food Quality Protection Act of August 3, 1996) should be retained at 10X when assessing the risks posed by the use of this pesticide. This report replaces the previous

report of the FQPA Safety Factor Committee dated March 30, 1999 (HED Doc. No. 013288).

I. HAZARD ASSESSMENT

(*DRAFT Memorandum:* D. Anderson and E. Mendez to W. Hazel dated November 17, 1999)

On September 16, 1999, the HED Hazard Identification Assessment Review Committee (HIARC) reviewed additional data provided on ventral prostate weight from perinatal studies with vinclozolin. On November 4, 1999, the HIARC evaluated the literature data on potential effects on the brain and other potential neurotoxic effects from perinatal testing of substances, including vinclozolin, that have androgenic and antiandrogenic effects on exposed offspring.

1. Comprehensive Evaluation of the Open Literature

The HIARC considered an evaluation of the currently available literature related to androgenic activity with compounds like vinclozolin (a total of 89 published articles - including reviews and research articles). The published literature indicates that there is sufficient evidence that compounds like vinclozolin that may disrupt the neuroendocrine system through their anti-androgenic properties can cause significant changes in the morphological and biochemical development of the nervous system. The effects of these changes can be monitored at the molecular level by use of immunocytochemistry, fluorescent *in situ* hybridization (FISH), mobility shift assays, or transcription assay techniques. And at the organism level, behavioral tests can be conducted to ascertain the effects of anti-androgen treatment on learning and behavior (Memorandum: E. Mendez to J. Rowland and P. Wagner dated October 27, 1999).

2. Adequacy of the Toxicology Database

The HIARC concluded that a developmental neurotoxicity study (DNT) in rats with an expanded protocol is required due to concern for the anti-androgenic properties of vinclozolin and its metabolites. Since the current DNT study protocol may not be sufficient to detect the subtle findings reported in the open literature and to assess the relevant endpoints for vinclozolin, an expanded protocol is required.

3. Determination of Susceptibility

Prenatal studies in rats demonstrate enhanced susceptibility of rat fetuses as compared to maternal animals following *in utero* exposure to vinclozolin. In the developmental toxicity study in rats, anti-androgen related effects, such as reduced anogenital distance (AGD), areola/nipple development, and ventral prostate weight decrease, occur at lower dose levels than effects on mothers dosed at the same dose levels and time period. There was no indication of increased susceptibility to young rabbits following *in utero* exposures or in the prenatal dermal developmental toxicity studies in rats. Additionally, in the 2-generation reproduction study in rats, effects in the offspring were observed only at or above dose levels causing parental

toxicity.

II. EXPOSURE ASSESSMENT

1. Dietary Exposure Considerations
(*Correspondence:* W. Hazel to B. Tarplee dated November 18, 1999)

Tolerances are established for the combined residues of the fungicide vinclozolin and its metabolites containing the 3,5-dichloroaniline moiety in or on several raw agricultural commodities at levels ranging from 1 to 25 ppm (40 CFR§180.380). Tolerances are currently undergoing tolerance reassessment for the HED Chapter of the RED. High consumption food items for infants and children include strawberries and stone fruit, however these tolerances will no longer be supported after January 2000. New uses are currently proposed for canola and snap beans. Codex MRLs are established for many commodities and are similar or identical to U.S. tolerances.

Although monitoring data are available for vinclozolin, these data are not considered to be useful for risk assessment since only parent vinclozolin was analyzed. Field trial data on vinclozolin and its metabolites containing the 3,5-dichloroaniline moiety are available on all crops. Virtually all of the field trial samples bore detectable residues ranging from 0.05-8.4 ppm. In addition to residue data, percent crop treated (%CT) and percent crop imported (%CI) data have been provided by the Biological and Economic Analysis Division (BEAD).

The HED Dietary Exposure Evaluation Model (DEEM) is used to assess the risk from acute and chronic dietary exposure to vinclozolin residues in food. These analyses will be performed using reassessed tolerance level residues and refined using the entire distributions of field trial data along with % CT and %CI data resulting in a less exaggerated representation of dietary food exposure resulting from the use of vinclozolin.

2. Drinking Water Exposure Considerations
(*Correspondence:* W. Hazel to B. Tarplee dated November 18, 1999)

The environmental fate data base for vinclozolin is adequate for the characterization of drinking water exposure. The data indicate that parent vinclozolin and degradates are potentially mobile and can be persistent depending upon the environmental conditions. Therefore, EFED concluded that vinclozolin has the potential to contaminate surface and ground water. Additionally, residues originating from parent vinclozolin may accumulate from year to year and be available for rotational crop uptake.

No groundwater studies are available for vinclozolin. Groundwater EECs were estimated using the SCI-GROW model. This estimate represents both acute and chronic exposure and were recommended for use in the risk assessment.

No available surface water monitoring data are available for vinclozolin. Surface water EECs were estimated using GENEEC (version 1.2). Peak and 56-day mean exposure estimates were generated using the maximum application rates for strawberries, peaches, and turf. Estimates of drinking water concentrations of vinclozolin derived from surface water are currently being refined using PRZM/EXAMS. These estimates will include the proposed uses and the new application rates.

3. Residential Exposure Considerations
(*Correspondence:* J. Dawson to B. Tarplee dated November 18, 1999)

Although there are no registered residential uses for vinclozolin (direct application in residential areas is not allowed by current labeling), there is concern for non-occupational post-application exposure to infants and toddlers resulting from treated sod placed around the home or other recreational areas. Golf course greens and tees can also be treated which could result in exposure to the general public (low exposures for this scenario are anticipated).

The *Standard Operating Procedures For Residential Exposure Assessment* will be used as the basis for the risk assessment for vinclozolin. There are a number of dislodgeable foliar residue studies for vinclozolin including a turf study. Additionally, there is a Jazzercize exposure study on turf. These data will be used, where appropriate, to calculate residue concentrations and exposures over time instead of using the Agency default assumptions. These chemical-specific data were generated using maximum application rates which result in conservative exposure estimates.

III. RISK CHARACTERIZATION

1. FQPA Safety Factor Recommendation

The FQPA SFC recommended that the safety factor for protection of infants and children (as required by FQPA) should be retained at 10X for Females 13-50 and Infants and Children subpopulations when assessing acute dietary (where applicable) and short-/intermediate-term residential (non-occupational) exposures. The Committee also recommended that when assessing chronic dietary exposures, the safety factor should be retained at 10X for All Population Subgroups.

2. Rationale for Requiring the FQPA Safety Factor

The FQPA SFC concluded that a safety factor is required because:

< there is evidence of increased susceptibility following *in utero* exposure to vinclozolin

in the prenatal developmental toxicity study in rats; and

< a developmental neurotoxicity study in rats with an expanded protocol is required for vinclozolin due to concern for the anti-androgenic properties of vinclozolin and its metabolites.

3. Application of the Safety Factor - Population Subgroups / Risk Assessment Scenarios

When assessing Acute Dietary (if applicable)* and Short-/Intermediate-term Residential (Non-occupational) Exposures, the safety factor should be Retained at 10X for the Females 13-50 and for the Infants and Children Subgroups since an increase in susceptibility was observed following *in utero* exposure to rats in the developmental study (which could potentially occur after a single dose); and since there is a data gap for the developmental neurotoxicity study which could provide information relevant to all population subgroups and exposure durations.

*Since no appropriate dose/endpoint was identified by the HIARC for use in acute dietary risk assessments for Infants and Children, the safety factor is not applicable to this population subgroup at this time.

When assessing Chronic Dietary Exposure, the safety factor should be Retained at 10X for All Population Subgroups since there is concern for reproductive effects (seen in testes, sperm, epididymides, and ovaries) observed at one or mores doses in the chronic studies used to establish the chronic RfD. Additionally, there is a data gap for the developmental neurotoxicity study which could provide information relevant to all population subgroups and exposure durations.